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Carbon monoxide attenuates aeroallergen-induced inflammation in mice

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Chapman, Jeffrey T., Leo E. Otterbein, Jack A. Elias, and Augustine M. K. Choi. Carbon monoxide attenuates aeroallergen-induced inflammation in mice. *Am J Physiol Lung Cell Mol Physiol* 281: L209–L216, 2001.—Carbon monoxide (CO) generated by catalysis of heme by heme oxygenase is increased in the exhaled air of asthmatic patients. Based on recent studies demonstrating that asthma is an inflammatory disease associated with increased oxidants and that CO confers cytoprotection in oxidant-induced lung injury and inflammation, we sought to better understand the functional role of CO in asthma by using an aeroallergen model. Mice were sensitized to ovalbumin, challenged with aerosolized ovalbumin, and maintained in either CO (250 parts/million) or room air for 48 h. The differential effects of CO on bronchoalveolar lavage (BAL) fluid cell types were observed, with a marked attenuation of BAL fluid eosinophils in the CO-treated animals at 24 and 48 h. A marked reduction of the proinflammatory cytokine interleukin-5 was observed in the CO-treated mice, with no significant changes for other proinflammatory cytokines. These differential effects of CO were also observed with leukotrienes (LTs) and prostaglandins in that CO significantly decreased BAL fluid PGE_2, and LTB_4 but exerted negligible effect on thromboxane B_2 or $LTC_4/D_4/E_4$. Our data suggest a putative immunoregulatory role for CO in aeroallergen-induced inflammation in mice.

heme oxygenase; asthma; eosinophils; ovalbumin; cytokines

ASTHMA IS A COMPLEX INFLAMMATORY DISEASE affecting 10 million people in the United States, with a cost exceeding $6 billion per year (37, 64). During the past decades, much work has focused on the mechanism(s) by which various leukocytes and cytokines mediate the inflammatory process often observed in asthma. Numerous studies of bronchoalveolar (BAL) fluid and biopsies from asthmatic airways have shown an increase in CD4-positive T cells and their T helper cell (Th) type 2 (Th2)-like products interleukin (IL)-4, IL-5, IL-13, and eotaxin (15, 27, 53, 55). Activated CD4 T lymphocytes and their cytokine products are critical in initiating and maintaining inflammation and bronchial hyperreactivity (3, 6, 7, 10, 14, 18, 68). Eosinophilic inflammation, driven by T-lymphocyte and Th2-like cytokines, is an important marker and possible mediator of inflammation in a number of human and animal studies (1, 2, 57). However, many recent studies (4, 8, 22) conflict regarding which cells and cytokines are essential, and robust cause-and-effect relationships are often difficult to establish.

Into this confusing milieu, oxidant stress has recently been introduced as playing an important role in the pathogenesis of inflammation in asthma. Patients with asthma have elevated plasma lipid peroxidation, a marker of systemic oxidative stress, reflecting an imbalance in prooxidant and antioxidant systems (54). Physician visits for asthma are positively correlated with ambient oxidants, with classic oxidants such as NO_2 and SO_2 worsening asthma pollution (16, 20). In addition, eosinophils are thought to mediate many of their effects through their ability to generate oxidizing species (19). Indeed, the respiratory burst of eosinophils generates several times as much superoxide and hydrogen peroxide as neutrophils (12).

Endogenous carbon monoxide (CO), generated via the enzyme heme oxygenase (HO), was first noted to be elevated in the exhaled breath of asthmatic patients by Zayasu et al. (67). Healthy control subjects had exhaled CO levels of 1.5 ± 0.1 parts/million (ppm), whereas mildly asthmatic patients had levels of 5.6 ± 0.6 ppm. Increased CO levels in the breath of asthmatic patients were further confirmed by other investigators (50). The increase in exhaled CO is reflective of increased HO-1 protein in the inflammatory cells of asthmatic patients (25). Many laboratories, including our own, have explored the role of HO-1 in oxidant lung injury and inflammation. Induction of endogenous HO-1 provides protection against oxidative stress in various in vivo and in vitro models (39, 42, 43, 66) including hyperoxia and lipopolysaccharide-induced tissue injury (9, 23, 24,

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29, 31, 38, 40, 41, 56, 65). Recent studies in our laboratory (47, 48) further demonstrate that an exogenously administered low concentration of CO confers cytoprotection in oxidant-induced lung injury, primarily via anti-inflammatory effects, suggesting CO as an important mediator of HO-1-induced cytoprotection. These findings led to the hypothesis that exogenous CO may also modulate the inflammatory response in an eosinophil-driven model of lung inflammation.

METHODS

Sensitization and challenge of mice. Female BALB/c mice age 8–10 wk were purchased from Harlan Sprague Dawley (Indianapolis, IN). The mice were housed in a pathogen-free facility at the Connecticut Veterans Affairs HealthCare System (West Haven, CT). Mice were sensitized as described by Kung et al. (35). Briefly, the mice received 20 μg of ovalbumin (Sigma, St. Louis, MO) and 2 mg of aluminum hydroxide gel (Intergen, Purchase, NY) intraperitoneally on *days 0* and *5*. On *day 12*, the mice were challenged by exposure to an aerosol of 0.6–1.0% ovalbumin in PBS for 10 min. Sham-challenged mice received an aerosol of PBS for 10 min. Aerosol challenge was carried out in a vented plastic chamber (18 × 14 × 8 cm). Aerosol particles 1–5 μm in diameter were created from an ultrasonic nebulizer (NE-U07, Omron, Vernon Hills, IL), directed into the plastic chamber, and vented to a fume hood.

CO exposure. CO exposures were performed as previously described (48) except for the following modifications. Animals were exposed in a sealed Plexiglas chamber that was continuously fed with medical-grade air and 250 ppm CO. The exchange rate of the chamber air was calculated to be two times per minute. CO concentration was continuously monitored within the chamber, and the chamber was vented to an approved fume hood.

Mice in the treatment group were exposed to 250 ppm CO for 2 h before aerosol challenge. The animals were briefly challenged in room air. After challenge, treated animals were exposed continuously to 250 ppm CO until death. Control animals were exposed only to room air for the duration of the experiments.

BAL. BAL was performed 24 and 48 h after aerosol challenge. The mice were anesthetized, and the lungs and heart were surgically exposed. The animals were exsanguinated by aortic transection. The trachea was cannulated, and the lungs were lavaged three times with 0.6-ml aliquots of PBS. Viable cells were counted with a hemocytometer. Smears were prepared by cytocentrifugation (Shandon, Pittsburgh, PA) at 400 rpm for 2 min and stained with HEMA 3 (Fisher Scientific, Hampton, NH). Differential cell counts on 200 cells/animal were enumerated based on morphology and staining profile.

Bone marrow and peripheral blood analysis. Forty-eight hours after ovalbumin challenge, bone marrow (BM) cells were collected from one femur according to the procedure described by Murali et al. (44). Briefly, one femur from each mouse was flushed with 1 ml of PBS. Red blood cell lysis was performed with PharM Lyse (PharMingen, San Diego, CA) according to the manufacturer's protocol. Smears were prepared by cytocentrifugation at 400 rpm for 2 min and stained with HEMA 3.

Peripheral blood (PB) was obtained by ventricular puncture 48 h after ovalbumin exposure. Red blood cell lysis, cytocentrifugation, and staining were performed on a 100-μl aliquot. Differential cell counts on 200 cells/animal were enumerated based on a morphology and staining profile for BM and PB cells.

Cytokine and eicosanoid assays. BAL fluid was centrifuged at 3,000 *g*, and the supernatant was stored at −70°C for later analysis. IL-1β, IL-4, IL-5, IL-10, eotaxin, tumor necrosis factor-α, interferon (IFN)-γ, monocyte chemoattractant protein-1, and macrophage inflammatory protein-1α protein levels in BAL supernatants were determined by ELISA (R&D Systems, Minneapolis, MN). Prostaglandin E_2, thromboxane B_2, leukotriene (LT) B_4, and $LTC_4/D_4/E_4$ eicosanoid levels in BAL supernatants were determined by ELISA (Amersham).

Statistical analysis. Data are means ± SE. Differences in measured variables between the experimental and control groups were assessed with Student's *t*-test. Statistical calculations were performed on a Macintosh personal computer with the StatView II statistical package (Abacus Concepts, Berkeley, CA). Significant difference was accepted at $P < 0.05$.

RESULTS

CO differentially attenuates ovalbumin-induced inflammation. BAL fluid was collected from sham- and ovalbumin-challenged animals at 24 and 48 h. Treated animals were exposed to a low concentration (250 ppm) of CO, whereas control animals were maintained in room air. Ovalbumin-challenged mice maintained in room air developed a fourfold increase in the number of total inflammatory cells in the BAL fluid at 24 h, with a further increase observed at 48 h (Fig. 1). In contrast, ovalbumin-challenged animals that were maintained in CO exhibited a significant reduction in the number of BAL fluid cells at 48 h (from 25.1×10^4 to 13.0×10^4 cells/ml; $P = 0.0015$; Fig. 1). Sham-challenged mice maintained in room air exhibited a baseline number of BAL fluid cells that did not change at 24 or 48 h. CO exposure did not affect the number of BAL fluid cells in the sham-challenged mice at either time point.

Differential cell analysis of BAL fluid from animals before challenge and 24 and 48 h post-sham challenge revealed macrophages exclusively, which did not in-

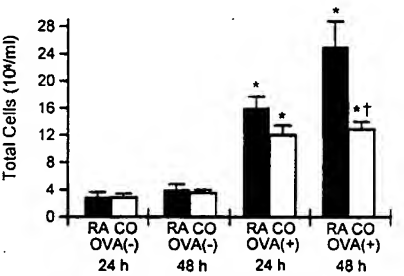

Fig. 1. Effects of exogenous carbon monoxide (CO) on bronchoalveolar lavage (BAL) fluid total cells. BAL fluid from ovalbumin-challenged [OVA(+)] and sham-challenged [OVA(−)] mice was obtained 24 and 48 h postchallenge. Treated animals received 250 parts/million (ppm) exogenous CO for 2 h before challenge and continuously thereafter. Control animals were maintained in room air (RA) for the duration of the experiment. *Significantly different from sham-challenged animals, $P < 0.05$. †Significantly different from animals maintained in RA, $P < 0.001$.

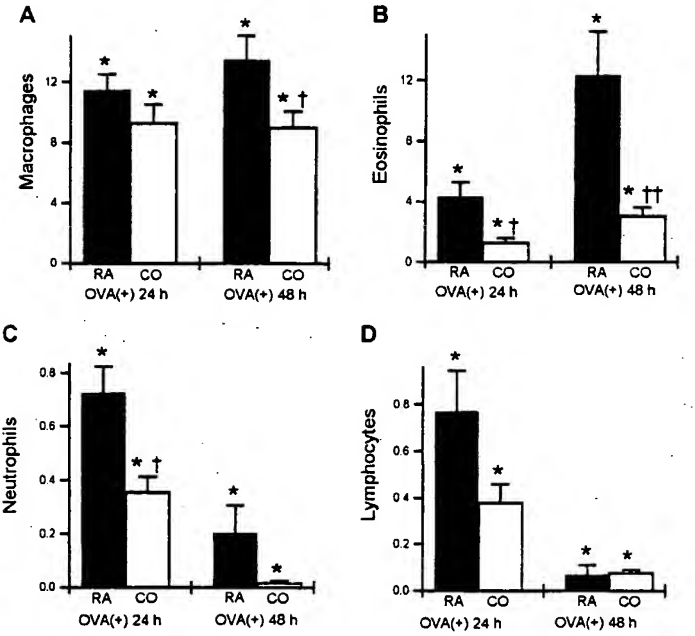

Fig. 2. Effects of exogenous CO on BAL fluid cell differential. BAL fluid from OVA(+) mice was obtained 24 and 48 h postchallenge. Treated animals received 250 ppm exogenous CO for 2 h before challenge and continuously thereafter. Control animals were maintained in RA for the duration of the experiment. Cell types quantified in BAL fluid were macrophages (*A*), eosinophils (*B*), neutrophils (*C*), and lymphocytes (*D*) and are expressed as no. of cells $\times 10^4$/ml. *Significantly different from sham-challenged animals, $P < 0.05$. Significantly different from animals maintained in RA: †$P < 0.05$; ††$P < 0.0007$.

crease at 24 or 48 h and which were unaffected by CO exposure (data not shown). However, ovalbumin-challenged animals demonstrated a robust increase at 24 h, with a further increase at 48 h (Fig. 2*A*). CO exposure caused a significant reduction at 48 h (from 13.7×10^4 to 9.8×10^4 macrophages/ml; $P = 0.0154$; Fig. 2*A*).

After ovalbumin challenge, a brisk increase in BAL fluid eosinophils was also observed at 24 h, with a further increase at 48 h (Fig. 2*B*). Interestingly, CO exposure caused a significant reduction at 24 h (from 4.3×10^4 to 1.3×10^4 eosinophils/ml; $P = 0.020$) and 48 h (from 12.3×10^4 to 3.0×10^4 eosinophils/ml; $P = 0.0007$). CO also differentially affected ovalbumin-induced BAL fluid neutrophils and lymphocytes (Fig. 2, *C* and *D*). Ovalbumin-challenged animals exhibited an increase in BAL fluid neutrophils at 24 h, which decreased at 48 h, whereas a significant reduction in BAL fluid neutrophils was observed in the CO-exposed animals at 24 h ($P = 0.0388$; Fig. 2*C*). CO exerted negligible effects on ovalbumin-induced BAL fluid lymphocytes (Fig. 2*D*). Eosinophils, lymphocytes, and neutrophils were absent from BAL fluid from sham-challenged animals (data not shown).

Effects of CO on BM and PB. To determine whether CO-induced attenuation of BAL fluid eosinophils was due to modulation of eosinophil production within the BM or eosinophil recruitment from the BM to the PB, differential cell counts of BM and PB eosinophils were examined. Forty-eight hours after ovalbumin challenge, BM and PB eosinophil percentages were not affected by CO exposure (Table 1).

Differential effects of CO on ovalbumin-induced proinflammatory cytokines. Numerous cytokine mediators, especially the Th2-like cytokines, have been implicated as essential in human asthma and animal models of eosinophilic inflammation. To understand the mechanism of the CO-induced reduction in BAL fluid eosinophilia, we determined the levels of some of these mediators in our model. IL-5, IL-4, and eotaxin were analyzed in the BAL supernatant. A significant reduction in IL-5 was observed in the ovalbumin-challenged mice in the presence of CO at 24 h ($P = 0.032$)

Table 1. *Eosinophil percentages in bone marrow and peripheral blood*

	OVA(+)	*P* Value
Bone marrow		
Air	4.6 ± 0.6	0.26
CO	5.7 ± 0.8	
Peripheral blood		
Air	16.5 ± 1.1	0.23
CO	14.5 ± 1.1	

Values are mean percentages ± SE. Bone marrow and peripheral blood from ovalbumin-challenged [OVA(+)] mice were obtained 48 h postchallenge. Treated animals received 250 parts/million exogenous carbon monoxide (CO) for 2 h before challenge and continuously thereafter. Control animals wer maintained in room air for the duration of the experiment.

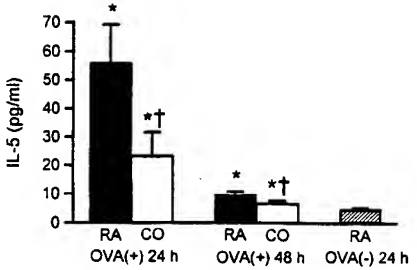

Fig. 3. Effects of exogenous CO on BAL fluid interleukin (IL)-5. BAL fluid from OVA(+), and OVA(−) mice was obtained 24 and 48 h postchallenge. Treated animals received 250 ppm exogenous CO for 2 h before challenge and continuously thereafter. Control animals were maintained in RA for the duration of the experiment. *Significantly different from sham-challenged animals, $P < 0.05$. †Significantly different from animals maintained in RA, $P < 0.03$.

and 48 h ($P = 0.022$; Fig. 3), whereas IL-4 increased to a peak at 24 h in the ovalbumin-challenged mice but was unaffected by CO exposure (Table 2). Eotaxin did not change significantly after ovalbumin challenge with or without CO exposure (Table 2). No significant changes at either 24 or 48 h were observed for the other proinflammatory mediators (Table 2).

Effects of CO on eicosanoid mediators. Eicosanoid products from various cellular sources are import mediators of eosinophilic inflammation in human asthma and aeroallergen-induced animal models. We measured eicosanoid mediators in sham- and ovalbumin-challenged mouse BAL supernatants to determine whether CO affected their production. All mediators were absent in sham-challenged BAL fluid, and PGE_2, LTB_4, and thromboxane B_2 increased with ovalbumin challenge. Only PGE_2, and LTB_4 were significantly reduced by CO exposure and only at 48 h (Fig. 4, *A* and *B*).

DISCUSSION

We have shown that exogenous administration of low concentrations of CO can ameliorate aeroallergen-induced inflammation. Specifically, we have shown that exogenous CO markedly reduces BAL fluid eosinophilia, a general marker of inflammation in asthma. After ovalbumin challenge, BAL fluid total cell counts increased fourfold at 24 h and peaked at a sixfold increase by 48 h. Eosinophils and macrophages comprised the overwhelming majority of cells recruited into the BAL fluid. Continuous CO exposure markedly decreased BAL fluid eosinophils and also caused a slight decrease in macrophage extravasation.

Although recent studies (47, 48, 58, 62) have shown beneficial effects of CO, it is classically thought of as a deleterious molecule. CO is known to have significant cardiovascular, neurological, and oxygen delivery effects and can even lead to death at high concentrations. Several studies, however, document the relative safety of CO doses similar or equivalent to ours. The concentration of CO (250 ppm) used in our studies is much lower than the level used in humans (3,000 ppm) during measurement of the pulmonary diffusing capacity of CO, a standard pulmonary function test, although our studies involved continuous CO exposure. Furthermore, in extensive studies by Stupfel and Bouley (59), long-term (2-yr) exposure of rodents to low levels of CO (500 ppm) exerted no significant alterations in physiological or biochemical parameter. Jones et al. (33) demonstrated that rats, dogs, and monkeys exposed to 200 ppm CO continuously for 90 days developed no noticeable toxic signs. Petajan et al. (52) studied the conduction velocity of the ventral caudal nerve and the visual

Table 2. *Effects of exogenous CO on BAL fluid cytokine levels*

Cytokine	Exposure	OVA(−) for 24 h	OVA(+) for 24 h	*P* Value	OVA(+) for 48 h	*P* Value
IL-4	RA	0 ± 0.0	113 ± 27.0	0.92	5.89 ± 0.18	0.41
	CO		107 ± 58.1		5.70 ± 0.75	
Eotaxin	RA	7.07 ± 0.55	10.7 ± 1.37	0.904	10.5 ± 0.63	0.45
	CO		10.4 ± 0.64		11.1 ± 0.55	
IFN-γ	RA	7.35 ± 8.89	7.27 ± 0.47	0.003	5.72 ± 0.45	0.31
	CO		2.02 ± 2.02		4.85 ± 0.75	
IL-10	RA	15.0 ± 0.44	14.8 ± 0.33	0.93	14.4 ± 0.35	0.78
	CO		14.8 ± 0.37		14.3 ± 0.39	
IL-1β	RA	3.67 ± 0.00	9.02 ± 0.78	0.32	5.73 ± 0.39	0.29
	CO		7.50 ± 1.38		4.79 ± 0.87	
TNF-α	RA	18.3 ± 5.95	11.7 ± 1.26	0.85	11.5 ± 0.32	0.86
	CO		12.0 ± 0.27		11.5 ± 0.17	
RANTES	RA	3.67 ± 0.00			5.13 ± 0.23	0.63
	CO				5.05 ± 0.27	
MCP-1	RA	2.64 ± 0.35			2.88 ± 0.27	0.024
	CO				2.06 ± 0.20	
MIP-1α	RA	3.15 ± 0.07			3.86 ± 0.18	0.052
	CO				3.39 ± 0.14	

Values are means ± SE in pg/ml. BAL, bronchoalveolar lavage; IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; RANTES, regulated on activation normal T cell expressed and secreted; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RA, room air. BAL fluid from OVA(+) and sham-challenged [OVA(−)] mice was obtained 24 and 48 h postchallenge. Treated animals received 250 parts/million exogenous CO for 2 h before challenge and continuously thereafter. Control animals were maintained in RA for the duration of the experiment.

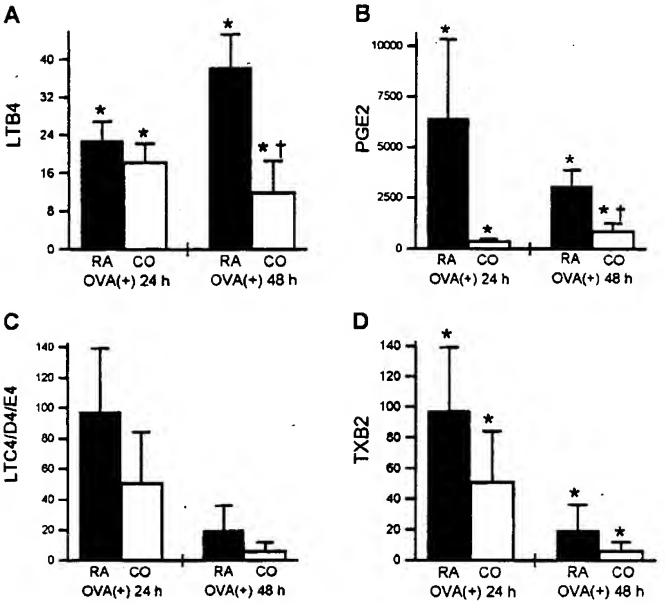

Fig. 4. Effects of exogenous CO on BAL fluid eicosanoid levels. BAL fluid from OVA(+) mice was obtained 24 and 48 h postchallenge. Treated animals received 250 ppm exogenous CO for 2 h before challenge and continuously thereafter. Control animals were maintained in RA for the duration of the experiment. Eicosanoids quantified in BAL fluid were leukotriene (LT) B_4 (*A*), PGE_2 (*B*), $LTC_4/D_4/E_4$ (*C*), and thromboxane B_2 (TxB_2; *D*) and are expressed in pg/ml. *Significantly different from sham-challenged animals, $P < 0.05$. †Significantly different from animals maintained in RA, $P < 0.05$.

cortical evoked potential in rats that were exposed to 1,500 ppm CO. They failed to observe any changes from baseline function until the carboxyhemoglobin (COHb) level exceeded 60%, far in excess of the levels in our studies. Kanten et al. (34) demonstrated that adult rats exposed to inhaled 500 ppm CO for 48 h developed COHb levels of 35.5%. This level of COHb caused the heart rate to increase by 20% and the systolic blood pressure to fall by 20%. Although the physiological changes are significant, their importance in terms of our model is unclear.

In human asthma, BAL fluid eosinophilia correlates with asthma severity and epithelial damage (2). In mice, BAL fluid eosinophilia correlates with airway hyperresponsiveness (AHR) in a study of aeroallergen-induced inflammation (21). However, some animal studies contradict these observations. Airway hyperresponsiveness does not always accompany BAL fluid eosinophilia and can be strain and dose dependent (13). In IL-5-overproducing transgenic mice, eosinophilia can occur without AHR (36). AHR occurs in the absence of BAL fluid eosinophilia in an IL-5-deficient murine model and in a toluene diisocyanate model of asthma (11, 26). Despite a lack of consensus, BAL fluid eosinophilia is still thought to be at least a marker of asthma inflammation, if not a cause of AHR.

The precise mechanism(s) by which CO mediates this reduction in inflammation is not clear. Eosinophils arise within the BM from progenitor cells that progressively increase after aeroallergen challenge (28). Six hours after challenge, BM eosinophils decrease, but by 72 h, a significant increase is seen (46). Decreased eosinophil production within the BM or decreased recruitment from the BM to the PB could account for our observations. Our data demonstrate no difference in BM eosinophils at 48 h, indicating that CO exposure does not affect eosinophil production or egress at that time point. PB eosinophils are known to increase in response to aeroallergen challenge (46). We did not observe differences in PB eosinophilia at 48 h, again suggesting that large changes in eosinophil trafficking are not accounting for the reduced inflammation. However, small changes in eosinophil sequestration within the lung over 48 h may account for the robust anti-inflammatory affects of CO.

The Th2-like ILs are pleiotropic molecules that coordinate eosinophilic inflammation. Il-5 has been shown to be critical for chemoattraction, activation, and survival of eosinophils in aeroallergen models. Exogenous CO significantly reduced BAL fluid IL-5 at 24 h, and levels returned to near baseline by 48 h. CO effects on IL-5 occurred in the absence of other cytokine changes. This speaks to a specific rather than a global anti-inflammatory effect of CO on the respiratory system. Reduced IL-5 at 24 h likely accounts for the large attenuation of BAL fluid eosinophilia seen at peak inflammation. The IL-5 source is unclear in this model. Cells known to produce IL-5 in the lung include Th2-like lymphocytes, mast cells, basophils, eosinophils, and epithelial cells.

Recently, other investigators have discovered links between Th1 ILs and the regulation of allergic inflam-

mation. Specifically, IFN-γ has been shown to be elevated by inhaled antigen and to reduce BAL fluid eosinophilia (5). However, in the present studies, IFN-γ was not increased in ovalbumin-challenged animals compared with sham-challenged animals. This may reflect our single-day nebulized ovalbumin challenge rather than prolonged and repeated challenges. A reduction in IFN-γ in the CO-exposed animals at 24 h (statistically significant) is interesting; however, the physiological significance of this effect is not clear given the lack of increase in the air-exposed animals.

Exogenous CO also affected eicosanoid mediators. LTB_4 was significantly reduced at 48 h. LTB_4 is a potent proinflammatory mediator that is increased in the BAL fluid of asthmatic patients (63). It has a wide variety of biological effects, including stimulation of neutrophil chemotaxis (49), and its reduction may account for the trend toward reduced neutrophils at 48 h. Interestingly, neutrophils were significantly reduced at 24 h without a concomitant change in LTB_4, suggesting another mechanism. Exogenous CO also reduced the amount of BAL fluid PGE_2. Curiously, PGE_2 has been shown to have both pro- and anti-inflammatory attributes. It attenuates allergen-induced sputum eosinophils in asthmatic patients (17) but enhances their survival in vitro (51). Although the source of eicosanoid mediators is unclear in our model, other investigators (45) have demonstrated that either exogenous CO or enhanced expression of HO-1 directly inhibits guinea pig mast cell activation. In addition, a recent study by Jia et al. (32) has demonstrated that HO-1 can protect against antigen-induced airway inflammation. In their rat model of antigen-induced inflammation, HO-1 induction inhibited extravasation of plasma into the trachea, main bronchi, and segmental bronchi. It is tempting to speculate that the anti-inflammatory effects of HO-1 in this model of antigen-induced inflammation are mediated by CO.

Although our studies point to modulation of IL-5 as a possible mediator of CO effects, others have investigated the role of intercellular adhesion molecule (ICAM)-1 in HO-1-induced protection. ICAM-1 has been shown to be important in the adhesion of human eosinophils to airway epithelial cells (30). Its blockade results in fewer BAL fluid eosinophils in a guinea pig model of allergen-induced asthma (60). Wagener et al. (62) investigated the effects of HO-1 on ICAM-1 expression on endothelial cells. They found that specific induction of HO-1 inhibited ICAM-1 and that blocking HO-1 expression with antisense oligonucleotides enhanced ICAM-1 expression. In addition, we speculate that CO could mediate its anti-inflammatory effects in this ovalbumin-induced model of inflammation via upstream signaling pathways such as the mitogen-activated protein (MAP) kinase pathway. In view of a recent report by Underwood et al. (61) that inhibition of p38 MAP kinase significantly inhibited inhaled ovalbumin-induced airway eosinophilia and recent observations by our laboratory (47) that CO inhibited endotoxin-induced lung inflammation via the p38 MAP kinase pathway, it is plausible that CO inhibits ovalbumin-induced pulmonary eosinophil influx via the MAP kinase pathway.

Our findings show that exogenous CO ameliorates inflammation in an aeroallergen-induced model of asthma. This is congruent with previous studies by our laboratory (47, 48, 58) showing that HO-1 induction and exogenous CO administration can reduce oxidant-mediated damage and inflammation in other models of lung injury including hyperoxia, endotoxin, and xenotransplantation. In our model, CO does not appear to affect eosinophil production or egress of eosinophils from the BM. In addition, we have demonstrated that a specific reduction in IL-5 may be an important mechanism in the effects of CO. In view of a recent report (25) that HO-1 expression is upregulated in human asthma, producing endogenous CO, and that an increased CO level is observed in the exhaled breath of patients with asthma, our studies suggest that CO may serve as an important modulator of allergic inflammation in asthma.

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